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Influence of Large Amounts of Nitrogen on Nonirrigated and Irrigated Soybean

Jeffery D. Ray,* Larry G. Heatherly, and Felix B. Fritschi

ABSTRACT

Nitrogen supplied by N_2 fixation to soybean [*Glycine max* (L.) Merr.] may not be sufficient to maximize yield. Field studies were conducted in 2002, 2003, and 2004 on Sharkey clay soil (very-fine, smectitic, thermic Chromic Epiaquert) at Stoneville, MS (33°26′ N lat). The objective was to determine the effect of high rates of N applied as a replacement for N_2 fixation in nonirrigated and irrigated environments. Eight cultivars ranging from Maturity Group II to IV were planted on 17 Apr. 2002, 2 Apr. 2003, and 25 Mar. 2004. Not all cultivars were evaluated in all 3 yr. Glyphosate herbicide was used in all 3 yr and a non-glyphosate herbicide treatment was applied in 2002. Cultivars grown in 2003 were also evaluated under an application of 21.3 kg ha^{-1} of Mn. All cultivar, herbicide, and Mn treatments were evaluated in irrigated and nonirrigated environments with fertilizer N (PlusN treatment) or without fertilizer N (ZeroN treatment). In the PlusN treatment, granular NH_4NO_3 was surface applied at soybean emergence at rates of 290 kg ha^{-1} in 2002, 310 kg ha^{-1} in 2003, and 360 kg ha^{-1} in 2004. When analyzed over all management practices (years, cultivars, herbicide, and Mn treatments), the PlusN treatment resulted in significantly decreased ureide concentration (57.2 and 53.5% reduction) and significantly increased biomass accumulation (14.1 and 16.7%), N accumulation (12.8 and 28.1%), and seed yield (7.7 and 15.5%) for the irrigated and nonirrigated environments, respectively. The majority of the yield increase in each environment resulted from increased number of seed (9.5% irrigated and 16.2% nonirrigated). These results confirm the sensitivity of N_2 fixation to drought and indicate that N_2 fixation may limit yield of soybean grown in both irrigated and nonirrigated environments of the midsouthern USA, and that N_2 fixation deficiencies occur before the beginning of processes that determine number of seed.

Water deficit stress (drought) is difficult to define and quantify because the magnitude of its effect depends on numerous crop (species, cultivar, phenology, etc.) and environmental factors (intensity, duration, evaporative demand, etc.), as well as their interactions. A deficit of certain intensity and duration may have differing effects on crop performance depending on the stage of development at which it occurs. Nonetheless, there is wide consensus that most plant physiological processes are unaffected by water deficits until >60% of the available soil water has been lost (Weisz et al., 1994; Sadras and Milroy, 1996). An exception to this generalization is N_2 fixation in soybean which has been shown to be more sensitive to water deficits than other physiological processes such as transpiration, photosynthesis, and biomass accumulation (Sinclair, 1986; Durand et al., 1987; Sinclair et al., 1987; Kirda et al., 1989; Djekoun and Planchon, 1991; Serraj et al., 1999). Increased sensitivity to water deficits is primarily concluded from experiments showing that N_2 fixation begins to respond (decline) at greater soil water contents than other physiological processes. The implication of this sensitivity is that even relatively limited water deficits can affect N accumulation and yield.

Purcell and King (1996) hypothesized that the uptake and assimilation of soil N was less sensitive to water deficits than was N_2 fixation, and that N fertilizer might ameliorate the effects of drought. They evaluated this hypothesis in a 1-yr field experiment using one cultivar (Hutcheson) in irrigated and nonirrigated (drought) plots with split applications of 224 kg N ha^{-1} at V6 and 112 kg N ha^{-1} at full bloom (R2) (stages according to Fehr et al., 1971). They found that N fertilizer applied to nonirrigated plots increased yield by about 18% over nonirrigated plots without fertilizer, but saw no effect of added fertilizer in irrigated plots. They concluded that N fertilizer applications do result in increased water deficit (drought) tolerance. Hutcheson was further examined by Purcell et al. (2004) in a 2-yr experiment of similar design to that reported by Purcell and King (1996). As with the previous experiment, yields of Hutcheson were increased in the nonirrigated treatment (9–25% increase), but unlike in the previous experiment, yields were also increased in the irrigated treatment by 9 to 15%.

Over the years, numerous studies have been conducted examining the application of N to soybean. In most cases these have been application of low rates of "Starter N." However, soybean grown on most soils does not respond to low rates (25–35 kg N ha^{-1}) of preplant N fertilization (Johnson, 1987; Varco, 1999; Hoeft et al., 2000; Heatherly et al., 2003; Scharf and Wiebold, 2003). The exceptions cited by Johnson (1987) were applications made to soils that were somewhat poorly drained, were low in organic matter, and/or were strongly acid below the plow layer. Other significant responses have been observed in soils with low residual soil nitrate (Lamb et al., 1990) or in situations where inorganic N is temporarily immobilized by soil microorganisms decomposing wheat straw (Whitney, 1997). In most cases, N fertilization of soybean is an unnecessary expenditure (Varco, 1999; Hoeft et al., 2000). Additionally, concentrations of N surrounding soybean roots can delay or impede nodulation (Harper and Gibson, 1984; Gibson and Harper, 1985), and thus reduce N_2 fixation.

Larger amounts of N fertilizer (100 to >500 kg ha^{-1}) have been applied to soybean with effects on yield rang-

Jeffery D. Ray, Research Geneticist, Larry G. Heatherly, Research Agronomist, and Felix B. Fritschi, Research Physiologist, USDA-ARS, Crop Genetics and Production Research Unit, P.O. Box 345, Stoneville, MS (662-686-3036). Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture and does not imply approval or the exclusion of other products that may also be suitable. Received 13 Jan. 2005. *Corresponding author (jray@ars.usda.gov).

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Abbreviations: DAP, days after planting; DTM, days to maturity; ESPS, early soybean production system; MG, maturity group.

Table 1. Description of factors analyzed over the 3 yr of a fertilizer N study with soybean at Stoneville, MS. Although some factors varied from year to year, all were replicated four times and were evaluated with and without fertilizer N (PlusN and ZeroN treatments).

Management practices (random effects)†					Fixed effects‡	
MG§	Cultivar	Year grown¶	Herbicide††	Added Mn‡‡	Irrigation§§	Fertilizer nitrogen¶¶
II	A 2703	2002	G, NG	N	N, I	P, Z
III	A 3702	2003, 2004	G	Y, N	N, I	P, Z
IV	A 4702	2002, 2003, 2004	G, NG	Y, N	N, I	P, Z
IV	AP 4882	2002	G, NG	N	N, I	P, Z
III	DK 3964	2003, 2004	G	Y, N	N, I	P, Z
IV	HBK 4820	2003	G	Y, N	N, I	P, Z
IV	HBK 4920	2004	G	N	N, I	P, Z
II	Jack	2002	G, NG	N	N	P, Z

† In the text, cultivar, year, herbicide and Mn treatments are collectively referred to as "Management Practices" which were treated statistically as random effects.
‡ Irrigation environment and nitrogen treatment were treated statistically as fixed effects.
§ Maturity Group classification.
¶ Year(s) of the study when the cultivar was evaluated.
†† Herbicide treatments, G = glyphosate all 3 yr, NG = non-glyphosate in 2002.
‡‡ Whether or not the cultivar was grown with the additional treatment of 23.1 kg ha^{-1} of Mn in 2003, Y = Yes and N = No.
§§ Irrigation environment under which the cultivar was grown, *N* = nonirrigated and I = irrigated.
¶¶ Added N, P = PlusN treatment (290 kg ha^{-1} in 2002, 310 kg ha^{-1} in 2003, and 360 kg ha^{-1} in 2004), Z = ZeroN treatment (no fertilizer N).

ing from no significant effect to significant increases (Lyons and Earley, 1952; Weber, 1966; Sorensen and Penas, 1978; Purcell and King, 1996; Gutierrez-Boem et al., 2004; Purcell et al., 2004). Differences in yield responses may be influenced by such factors as the soil water status during a specific experiment and the timing of the N application. For example, most positive responses to fertilizer N also report an increase in seed number per square meter with applied N at or before flowering, whereas applications of N postflowering are reported to have no effect on seed number per square meter or on yield. If soil water content becomes too low for too long, the effect may be so severe as to mask any beneficial responses to applied N. However, if less than optimal irrigation is applied, there may be room for applied N to overcome yield reductions caused by the sensitivity of N_2 fixation to mild water deficit stress.

Nitrogen availability or nutrition may be limiting productivity of soybeans planted using the early soybean production system (ESPS; Heatherly, 1999a; Heatherly and Bowers, 1999) in the midsouthern USA, especially in the early vegetative period. This may limit production potential in the later reproductive period. Application of early-season N to soybean may overcome the possible negative effects of insufficient N from N_2 fixation. The primary objective of these experiments conducted at Stoneville, MS, in 2002, 2003, and 2004 was to determine the agronomic and physiologic ramifications of inorganic fertilizer N applied as a replacement for N_2 fixation to ESPS soybean plantings in nonirrigated and irrigated environments.

MATERIALS AND METHODS

The study summarized in this report consists of a wide range of experimental factors (environments, cultivars, and management practices). While each factor is detailed below, an overview is presented to help orient the reader. The primary objective of these experiments was to determine the ramifications of inorganic fertilizer N applied as a replacement for N_2 fixation to ESPS soybean plantings in nonirrigated and irrigated environments over different cultivars, herbicide treatments, Mn treatments, and years (Table 1). To accomplish this we compared the application of very high rates of N (PlusN treatment; 290 kg ha^{-1} in 2002, 310 kg ha^{-1} in 2003, and 360 kg ha^{-1} in 2004) to no applied N (ZeroN treatment) under irrigated and nonirrigated environments. The study was broadened with the additional comparison of a non-glyphosate herbicide to a glyphosate herbicide treatment in 2002 and an applied Mn (23.1 kg ha^{-1}) treatment in 2003 (Table 1). The non-glyphosate herbicide treatment in 2002 was in response to concerns that glyphosate herbicide might delay the onset of nodulation and thereby affect seed yield. However, since little effect was observed and as the vast majority of southern soybean (>90%, National Agricultural Statistics Service, 2004) is produced using glyphosate technology, the non-glyphosate treatment was dropped in subsequent years. The Mn treatment in 2003 was included because of the possibility that Mn might overcome some of the adverse effects of water deficit stress on N_2 fixation (Purcell et al., 2000; Vadez et al., 2000; Sinclair et al., 2003).

Over the 3 yr of the study, the effect of fertilizer N was examined on a total of eight different soybean cultivars ranging from MG II to MG IV (Table 1) although not all cultivars were used in every year. Years, cultivars, herbicide, and Mn treatments are collectively referred to as "Management Practices" in the discussions below. Statistical analysis (detailed below) was conducted across all management practices and irrigation environments to determine the effect of adding large amounts of fertilizer N to soybean. The wide diversity of conditions under which the effect of fertilizer N was evaluated allows for broad analysis and interpretation.

The field studies were conducted in 2002, 2003, and 2004 at the Delta Research and Extension Center at Stoneville, MS (33°26′ N lat), on Sharkey clay soil. Sharkey is the dominant soil series in the lower Mississippi River Valley alluvial flood plain, and comprises about 1.2 million ha in Arkansas, Kentucky, Louisiana, Mississippi, Missouri, and Tennessee (Pettry and Switzer, 1996). The pH at the study site ranged from 6.5 to 7.7, and P and K levels were in the high category (Varco, 1999; Heatherly and Elmore, 2004) and needed no supplementation.

Experiments in separate nonirrigated and irrigated environments were conducted each year, but they were within 200 m of each other and had a common, uniform soil type. The irrigated environment was in a different location each year, while each year's nonirrigated environment was in the same location. Each environment was located on a site that had a respectively common irrigation history (either irrigated or nonirrigated) the previous 10 yr, and soybean had been grown on both sites the previous 20 yr. A randomized complete block design with four replicates was used each year. Treatments were arrayed in a split-plot factorial arrangement, with cultivar as the main plot and N rate as the subplot. Treatments in the irrigated environment were randomly assigned to plots at the beginning of each year, whereas those in the nonirrigated environment were randomly assigned in 2002 and remained in the same plot location for 2003 and 2004. Row width was 0.5 m and seeding rate was approximately 16 seed m^{-1} of row. Plots were 4 m wide (8 rows) and 22 m long. All experiments were seeded into a stale seedbed (Heatherly, 1999b) that had been shallow tilled (<10 cm deep) with a disk harrow and spring-tooth cultivator the preceding fall. Glyphosate at 840 g a.i. ha^{-1} in 94 L of water ha^{-1} was applied preplant to each experimental site each year to kill existing weed vegetation.

Planting dates were 17 Apr. 2002, 2 Apr. 2003, and 25 Mar. 2004. Maturity group II ('A 2703' and 'Jack') and MG IV ('A 4702' and 'AP 4882') cultivars were used in 2002, whereas MG III ('DK 3964' and 'A 3702') and MG IV (A 4702 and 'HBK 4820' in 2003 and A 4702 and 'HBK 4920' in 2004) cultivars were used in 2003 and 2004 (Table 1). The MG II cultivars (110 d to maturity [DTM]) and MG III cultivars (120 DTM) were chosen for their short growing season, whereas the MG IV cultivars (133 DTM in 2002, 138 DTM in 2003, and 145 DTM in 2004) were chosen to represent a normal growing season length in the ESPS. Cultivars were chosen based on regional variety trial results, use patterns by producers, and recency of release. The April planting dates and early-maturing cultivars are the key elements of the ESPS in the midsouthern USA (Heatherly, 1999a). Seed were treated with mefenoxam [(*R*)-2-{2,6-(dimethylphenyl)-methoxyacetylamino}-propionic acid methyl ester] fungicide at 0.11 g a.i. kg^{-1} seed before seeding each year.

Levels of N applied were 0 (ZeroN treatment) and 290 kg ha^{-1} (2002), 310 kg ha^{-1} (2003), and 360 kg ha^{-1} (2004) and are collectively referred to as the "PlusN" treatment. N was surface applied as granular NH_4NO_3 (340 g N kg^{-1} material) using a granular fertilizer applicator. The intent was to apply enough N fertilizer to support a seed yield of at least 4700 kg ha^{-1}, and this was accomplished each year. Cost of N was about \$1.04kg^{-1}, making cost per hectare \$302 in 2002, \$322 in 2003, and \$374 in 2004. Applications were made on 24 Apr. 2002, 16 Apr. 2003, and 22 Apr. 2004. Rainfall of >2 cm occurred 5, 8, and 10 d after N application in 2002, 2003, and 2004, respectively. Weather data presented in Table 2 were collected approximately 0.8 km from the experimental site by Delta Research and Extension Center personnel.

Plots were maintained weed free with postemergence applications of labeled herbicides. The irrigated environment was furrow irrigated using rollout vinyl pipe. Irrigation was initiated on 3 June 2002, 4 June 2003, and 9 June 2004, which was slightly before or slightly after beginning podset of all cultivars. Irrigations after the first application each year were applied whenever soil water potential at the 30-cm depth, as measured by tensiometers, decreased to about −50 kPa. Irrigation was continued through full seed (R6) of all cultivars.

The dry weight of aboveground biomass samples was determined for each observational unit (year, cultivar, herbicide and Mn treatments, irrigation environment and fertilizer N treatment) evaluated in the study (Table 1). Two biomass harvests were obtained in 2002, whereas one was made in each of 2003 and 2004. The first 2002 harvest occurred 54 d after planting (DAP) and corresponded to complete canopy closure in all plots. The second 2002 biomass harvest occurred at 83 DAP for A2703 and Jack and at 110 DAP for A4702 and AP4882. These sampling dates corresponded to approximately R6. One biomass sample was taken in 2003 and 2004 when each plot reached beginning seed fill (R5). In 2003, the harvests occurred at 69 DAP for A3702, 76 DAP for DK3964, and 79 DAP for A4702 and HBK4820. In 2004, the harvests occurred at 81 DAP for A3702, 83 DAP for DK3964, 88 DAP for A4702, and 104 DAP for HBK4920. All biomass samples consisted of side-by-side 1-m-long sections of each of the two center rows of each plot. The number of nodes and plant height were determined on two representative plants in the center two rows next to the biomass sampling area. Biomass samples were dried for at least 72 h at 60°C, weighed, and then ground in a Wiley mill (Model 4, Thomas Scientific, Swedesboro, NJ) using a 2-mm screen. A subsample of the ground material was further ground using a Cyclotec sample mill (FOSS, Eden Prairie, MN) with a 1-mm screen. Approximately 0.15 g of dried, finely ground sample was weighed into gel caps and the weight recorded. The samples were then analyzed for total N by combustion at an operating temperature of 950°C using a LECO FP428 Nitrogen Analyzer (LECO Corporation, St. Joseph, MI). The N analysis was conducted by the Agricultural Diagnostic Laboratory of the University of Arkansas (Fayetteville, AR).

An additional finely ground biomass subsample was analyzed for total ureide content using the following procedure. Approximately 0.12 g of the dried and ground biomass samples was extracted in 5 mL of 0.2 *M* NaOH for 30 min at 100°C. An aliquot of 1 mL was centrifuged at 10 000 × *g* for 5 min and the supernatant was transferred to a clean tube and stored at −20°C until analysis. Within 1 wk of extraction, samples were thawed at 4°C, centrifuged at 10 000 × *g* for 5 min, and 100-μL aliquots of extract were adjusted to 1 mL with ultrapure, deionized water and used for analyses. Colorimetric ureide determinations were conducted according to the alkaline-acid hydrolysis method of Vogels and van der Drift (1970) except that the reagent volumes were reduced to one fifth. Absorbance at 535 nm was measured on a μQuant spectrophotometer (Bio-Tek Instruments, Inc., Winooski, VT).

A field combine, modified for small plots, was used to harvest the four center rows of each plot between 5 and 30 Aug. 2002, between 28 July and 21 Aug. 2003, and between 3 and 23 Aug. 2004. All cultivars were harvested within 5 d of maturity (R8) each year. Harvested seed were weighed and adjusted to 130 g moisture kg^{-1}. Weights of two 100-seed samples per plot were also recorded and adjusted for moisture content. Calculations of number of seeds were made from yield and seed weight data.

To determine the overall effect of adding large amounts (>290 kg ha^{-1}) of fertilizer N to soybean, an analysis of variance [SAS PROC MIXED, Version 9 (Littell et al., 1996)] was conducted. In this analysis, years, Mn application, herbicide application, and cultivars were treated as a random effect, collectively referred to as management practices. Each management practice had two treatments, with or without fertilizer N (PlusN and ZeroN treatments), and was grown in two separate environments, irrigated or nonirrigated. Irrigation environment was treated as a fixed effect. Replicate plots (four each) of the management practices were considered subsamples of paired samples with or without fertilizer N. These analyses provided an estimate of the significance of an overall effect of applying large amounts of fertilizer N across a diverse range of environments, cultivars, and management practices, and as such was not designed to evaluate effects on individual cultivars or management practices. In this design, irrigation environments cannot be directly compared; however, the interaction between irrigation environment and fertilizer N can be used to evaluate the consistency of response to fertilizer N.

Table 2. Average daily maximum air temperatures (Max T) and total rain amounts during indicated periods of maturity group (MG) II, III, and IV soybean cultivars in 2002, 2003, 2004, and 30-yr normals for the same periods, at Stoneville, MS.

			Measured		Normal†	
MG	Period	Dates	Max T	Rain	Max T	Rain
			°C	mm	°C	mm
		2002				
II	Plant–R1	15 Apr.–17 May	28.3	80	25.5	151
	R1–R5	18 May–10 June	30.0	39	30.0	83
	R5–R6	11 June–8 July	31.7	103	32.8	90
IV	Plant–R1	15 Apr.–20 May	27.7	100	26.1	163
	R1–R5	21 May–27 June	31.1	30	31.1	127
	R5–R6	28 June–2 Aug	33.4	174	33.0	102
		2003				
III	Plant–R1	2 Apr.–10 May	26.7	100	24.2	179
	R1–R5	11 May–10 June	28.4	93	29.4	113
	R5–R6	11 June–15 July	31.4	181	32.8	109
IV	Plant–R1	2 Apr–13 May	26.7	123	24.4	192
	R1–R5	14 May–18 June	28.8	192	30.2	125
	R5–R6	19 June–28 July	32.4	68	33.0	120
		2004				
III	Plant–R1	25 Mar.–11 May	24.4	149	23.6	217
	R1–R5	12 May–11 June	29.7	165	29.6	112
	R5–R6	12 June–12 July	31.0	344	32.8	98
IV	Plant–R1	25 March–13 May	24.4	180	23.8	226
	R1–R5	14 May–14 June	30.4	133	29.9	112
	R5–R6	15 June–25 July	31.7	380	32.9	125

† 1964–1993 (Boykin et al., 1995).

RESULTS

Weather Conditions

Environmental conditions varied considerably across the 3 yr of the study when considered against the developmental periods of the different cultivars included in the study. Table 2 shows the average daily maximum air temperatures and total rain amounts during selected development stages (planting to flowering [R1], R1 to beginning seed fill [R5], and R5 to full seed [R6]) for each year and maturity group grown in the study. Additionally, the 30-yr normal maximum temperature and rainfall values for the same developmental periods are provided for reference (Table 2). Across all years and maturity groups the period from planting to R1 averaged 1.8°C warmer than the 30-yr normal, the period from R1 to R5 averaged 0.30°C cooler and the period from R5 to R6 averaged 0.95°C cooler. Rainfall data were less consistent than the temperature data but the trend was for less rainfall during the period of planting to R1 (average 66 mm decrease), about the same rainfall during R1 to R5 (average 3 mm decrease) and somewhat higher rainfall from R5 to R6 (average 101 mm increase).

Biomass and Plant Characteristics

Aboveground biomass samples were taken for each observational unit (year, cultivar, herbicide and Mn treatment, irrigation environment and fertilizer N treatment) in the study (Table 1). Two biomass samples were obtained in 2002 (at canopy closure and R6) and one each in 2003 and 2004 (at R5). Figure 1 shows a one-to-one graph of the biomass (g m^{-2}) of the PlusN treatment plotted against the ZeroN treatment over all management practices, N treatments, and irrigation environments of the 3 yr study. The data points represent 46

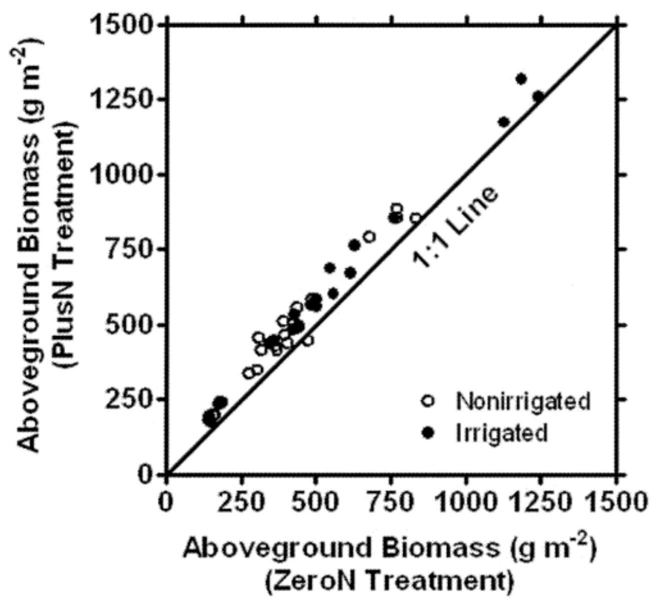

Fig. 1. One-to-one graph of aboveground biomass (g m^{-2}) for soybean in the ZeroN treatment (*x* axis) plotted against aboveground biomass in the PlusN treatment (*y* axis) across all management practices (years, cultivars, herbicide, and Mn treatments) and irrigation environments (irrigated and nonirrigated). Two biomass samples (54 days after planting [DAP] and approximately R6) were taken in 2002 and one biomass sample was taken at approximately R5 in 2003 and in 2004. The data points represent 46 paired (ZeroN vs. PlusN) samples (i.e., 92 observations with four replications each).

paired (ZeroN vs. PlusN) samples (i.e., 92 observations with 4 replications each). A random distribution on either side of the 1:1 line would indicate no effect. However, all but one observation appeared above the 1:1 line indicating fertilizer N (PlusN treatment) had a positive effect on aboveground biomass. Considered over all management practices (years, cultivars, herbicides, and Mn treatments) there was a significant ($P < 0.0001$)

Table 3. Fixed effects and components of error of the analysis of variance (Proc Mixed, SAS) for the parameters analyzed in this study. Years, Mn application, herbicide application, and cultivars were collectively analyzed as management practices (MP) and were treated as random effects. All management practices were grown in separate irrigation environments (irrigated and nonirrigated) which were treated as a fixed effect. Nitrogen treatments consisted of either large amounts (>290 kg ha^{-1}) of fertilizer N applied shortly after planting (PlusN) or no applied fertilizer N (ZeroN).

		Node number		Plant height§		R8 plant height		Biomass N concentration		Biomass ureide concentration	
				cm				g kg^{-1}		µmol g^{-1}	
Fixed effects	**df**	***F***	***P* > *F***	***F***	***P* > *F***	***F***	***P* > *F***	***F***	***P* > *F***	***F***	***P* > *F***
Irrigation†	1	0.00	0.9882	4.39	0.0420	8.32	0.0069	8.35	0.0006	11.42	0.0015
N	1	24.72	<0.0001	14.84	0.0004	33.99	<0.0001	28.31	<0.0001	76.57	<0.0001
Irrigation × N	1	1.62	0.2042	0.01	0.9257	0.50	0.4865	17.38	0.0001	4.47	0.0403
Components of error‡ (random effects)		Error	%	Error	%	Error	%	Error	%	Error	%
σ^2 (MP¶)		9.01	91.28	203.88	92.75	120.84	89.33	0.083	55.51	1.13	20.69
σ^2 (N × MP)		0.00	0.00	2.81	1.28	4.99	3.69	0.010	6.84	2.60	47.39
σ^2 (e)		0.86	8.72	13.13	5.97	9.45	6.99	0.056	37.65	1.75	31.93
Total		9.87	100.00	219.82	100.00	135.28	100.00	0.150	100.00	5.48	100.00
		Seed weight		**Biomass accumulation**		**Biomass N accumulation**		**Seed yield**		**Seed number**	
		mg seed^{-1}		g m^{-2}				kg ha^{-1}		seed m^{-2}	
Fixed effects	**df**	***F***	***P* > *F***	***F***	***P* > *F***	***F***	***P* > *F***	***F***	***P* > *F***	***F***	***P* > *F***
Irrigation	1	8.47	0.0064	2.54	0.1181	5.44	0.0243	33.13	<0.0001	13.21	0.0009
N	1	4.12	0.0505	90.15	<0.0001	90.11	<0.0001	187.67	<0.0001	116.66	<0.0001
Irrigation × N	1	0.90	0.3489	0.22	0.6426	3.04	0.0882	4.29	0.0463	1.67	0.2048
Components of error (random effects)		Error	%	Error	%	Error	%	Error	%	Error	%
σ^2 (MP)		2.56	84.61	71 710.0	93.62	58.40	88.93	445 861.0	89.81	252 416.0	90.16
σ^2 (N × MP)		0.18	5.88	0.0	0.00	0.03	0.05	1082.1	0.22	6931.9	2.48
σ^2 (e)		0.29	9.51	4888.4	6.38	7.23	11.02	49 508.0	9.97	20 631.0	7.37
Total		3.02	100.00	76 598.4	100.00	65.66	100.00	496 451.1	100.00	279 978.9	100.00

† ANOVA results for irrigation environment are shown for completeness. Direct comparison between irrigation environments was not statistically valid because of the structure of the experimental design.
‡ Proc Mixed of SAS was used and therefore components of variance are given instead of mean squares for random effects.
¶ MP, management practices which consists of years, herbicide treatments, Mn treatments, and cultivars.
§ Plant height at the time of the biomass sampling.

increase of 14.1% (517 vs. 589 g m^{-2}) in the PlusN treatment compared to the ZeroN treatment in the irrigated environment. Similar results were found in the nonirrigated environment although the increase was larger (16.7%, 394 vs. 460 g m^{-2}, $P < 0.0001$) with the addition of fertilizer N. However, there was no significant ($P = 0.6426$) difference in the effect of fertilizer N between irrigation environments (Table 3).

The number of nodes and plant height were determined for each aboveground biomass sample. As expected from the diverse group of cultivars and sampling dates, there was a wide range in values for both of these parameters (9.6–20.6 nodes plant^{-1} and 36.8–97.9 cm height, data not shown, ANOVA results in Table 3). When analyzed over all management practices and comparing the ZeroN and PlusN treatments in the irrigated environment, there was a small but significant ($P = 0.0109$) increase in the number of nodes plant^{-1} (14.7 vs. 15.1, Zero N vs. PlusN) and a small but significant ($P = 0.0127$) increase in plant height (61.9 vs. 63.9 cm, ZeroN vs. PlusN). Similar results were found in the nonirrigated environment (14.6 vs. 15.2 nodes plant^{-1}, Zero N vs. PlusN, $P < 0.0001$, and 53.0 vs. 55.0 cm, ZeroN vs. PlusN, $P = 0.0065$). Fertilizer N did not significantly affect nodes plant^{-1} ($P = 0.2042$) or plant height ($P = 0.9257$) between irrigation environments (Table 3). Final plant height was also measured at full maturity (R8, data not shown). When analyzed across all management practices, there were significant differences in plant height (R8) between the ZeroN and PlusN treatments in both the irrigated (71.7 vs. 75.0 cm, $P = 0.0011$) and the nonirrigated (60.3 vs. 64.6, $P < 0.0001$) environments. However, the effect of fertilizer N was not significantly ($P = 0.4865$) different between irrigation environments (Table 3).

Nitrogen Measurements

For each of the aboveground biomass samples, the N concentration (g kg^{-1}) was determined. In the irrigated environment, N concentration ranged from 30.4 to 39.5 g kg^{-1} in the ZeroN treatment and from 27.3 to 39.0 g kg^{-1} in the PlusN treatment (data not shown). In the nonirrigated environment, N concentration ranged from 23.1 to 37.4 g kg^{-1} in the ZeroN treatment and from 23.5 to 39.7 g kg^{-1} in the PlusN treatment (data not shown). When analyzed over all management practices in the irrigated environment, the PlusN treatment did not have a significantly ($P = 0.4295$) greater N concentration than the ZeroN treatment (34.0 vs. 33.7 g kg^{-1} N). However, there was a significant ($P < 0.0001$) difference between these treatments in the nonirrigated environment (32.8 g kg^{-1} N in the PlusN treatment and 29.7 g kg^{-1} N in the ZeroN treatment). The difference in response to fertilizer N between irrigation environments was significant ($P = 0.0001$, Table 3).

Biomass N was calculated from the N concentration (g kg^{-1}) and the aboveground biomass dry weight (g m^{-2}). Figure 2 shows a one-to-one graph of biomass N of

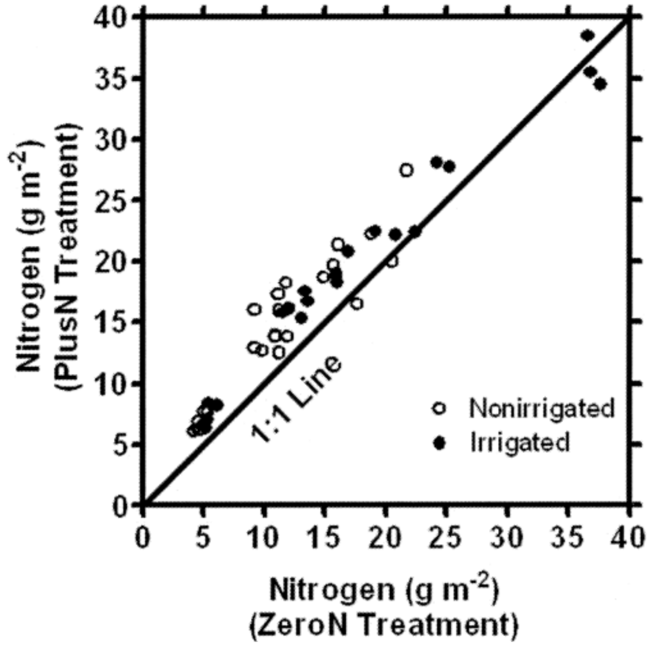

Fig. 2. One-to-one graph of Biomass N (g N m^{-2}) in the ZeroN treatment (*x* axis) plotted against Biomass N in the PlusN treatment (*y* axis) across all management practices (years, cultivars, herbicide, and Mn treatments) and irrigation environments (irrigated and nonirrigated). Biomass N was calculated from the N concentration (%N) and the aboveground biomass (g m^{-2}). The data points represent 46 paired (ZeroN vs. PlusN) samples (i.e., 92 observations with four replications each).

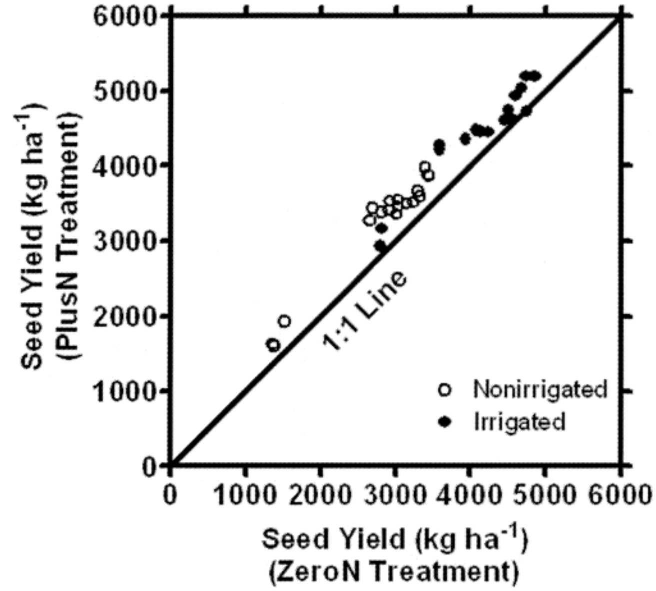

Fig. 3. One-to-one graph of seed yield (kg ha^{-1}) in the ZeroN treatment (*x* axis) plotted against seed yield in the PlusN treatment (*y* axis) across all management practices (years, cultivars, herbicide, and Mn treatments) and irrigation environments (irrigated and nonirrigated). The data points represent 35 paired (ZeroN vs. PlusN) samples (i.e., 70 observations with four replications each).

the PlusN treatment compared to the ZeroN treatment across all management practices, N treatments, and irrigation environments over all 3 yr of the study. The data points represent 46 paired (ZeroN vs. PlusN) samples (i.e., 92 observations with four replications each). All but five data points were above the one-to-one line (three irrigated and two nonirrigated). Four of the five data points below the one-to-one line were from the 2002 experiment and one was from 2004; otherwise there was little commonality between the values. When analyzed over all management practices in the irrigated environment, the PlusN treatment had a significant ($P <$ 0.0001) increase of 12.8% (17.2 vs. 19.4 g N m^{-2}) over the ZeroN treatment. In the nonirrigated environment, the addition of N (PlusN) resulted in a 28.1% (11.4 vs. 14.6 g N m^{-2}) increase in g N m^{-2}. In both irrigation environments, the addition of large amounts of fertilizer N resulted in greater accumulation of N, although the increase in the nonirrigated environment was much larger (12.8 vs. 28.1%). However, these differences between irrigation environments in response to fertilizer N were not significant (P = 0.0882, Table 3).

Ureide Measurements

In soybean, ureides (allantoin and allantoic acid) are the N transport molecules from the nodules to the leaves. In the leaves, ureides are metabolized and the N is released for incorporation into various compounds. It was expected that the large amounts of N applied in this study would inhibit nodulation and/or nodule function, resulting in an overall reduction of ureides. Across all biomass harvests, in the irrigated environment, ureide concentrations ranged from 2.0 to 5.3 μmol g^{-1} in the PlusN and from 3.3 to 12.8 μmol g^{-1} in the ZeroN treatment (data not shown). In the nonirrigated environment, ureide concentrations ranged from 1.0 to 3.8 μmol g^{-1} in the PlusN treatment and from 1.5 to 10.5 μmol g^{-1} in the ZeroN treatment (data not shown). Analysis over all management practices showed that in the irrigated environment the PlusN treatment significantly ($P < 0.0001$) decreased the ureide concentration by 57.2% (2.96 vs. 6.91 μmol g^{-1}). The effect was less (53.5%, 2.10 vs. 4.52 μmol g^{-1}) but still significant ($P <$ 0.0001) in the nonirrigated environment. There was a significant (P = 0.0403, Table 3) difference between irrigation environments in the effect of adding fertilizer N on ureides.

Yield and Yield Components

The ultimate measure of the effect of applied N is reflected in seed yield measurements. Comparison of the PlusN treatment to the ZeroN treatment is shown in the one-to-one graph of Fig. 3. The data points represent 35 paired (ZeroN vs. PlusN) samples (i.e., 70 yield observations with four replications each). All but one data point was above the one-to-one line, thereby highlighting the consistent increase in yield resulting from adding fertilizer N. For the irrigated environment, analysis over all management practices showed that seed yield in the PlusN treatment was significantly ($P < 0.0001$) increased (4184 vs. 4507 kg ha^{-1} or 7.71%) over the ZeroN treatment. Similarly, for the nonirrigated environment, analysis over all management practices showed that fertilizer N resulted in a significant ($P < 0.0001$) yield increase (15.53% increase, 2817 vs. 3255 kg ha^{-1}). The

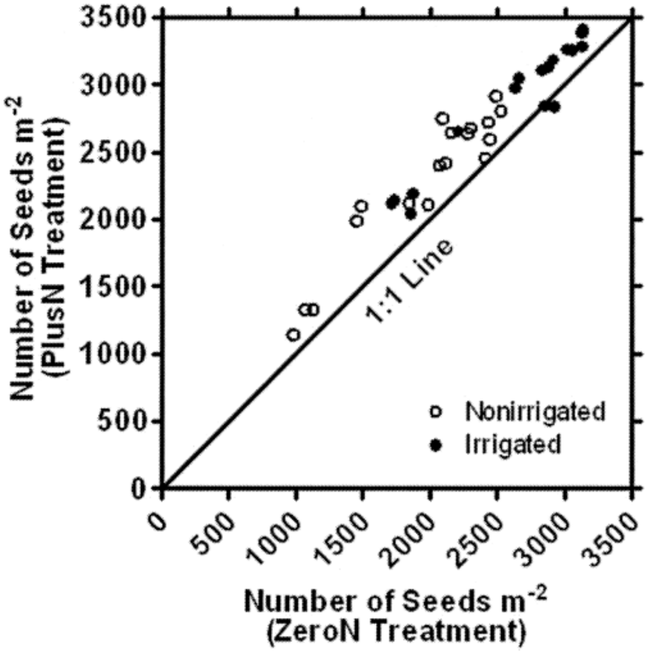

Fig. 4. One-to-one graph of the number of seed per square meter in the ZeroN treatment (*x* axis) plotted against the number of seed per square meter in the PlusN treatment (*y* axis) across all management practices (years, cultivars, herbicide, and Mn treatments) and irrigation environments (irrigated and nonirrigated). The data points represent 35 paired (ZeroN vs. PlusN) samples (i.e., 70 observations with four replications each).

response to fertilizer N between irrigation environments was significantly ($P = 0.0463$, Table 3) different.

The two primary determinants of seed yield are seed weight and seed number. Seed weight in the irrigated environment ranged from 142 to 210 mg seed^{-1} in the ZeroN treatment and from 135 to 200 mg seed^{-1} in the PlusN treatment (data not shown). In the nonirrigated environment, seed weight ranged from 121 to 182 mg seed^{-1} in the ZeroN treatment and from 123 to 167 mg seed^{-1} in the PlusN treatment (data not shown). Over all management practices in the irrigated environment, there was a small but significant ($P = 0.0456$) reduction of 2.19% in seed weight (158 vs. 162 mg seed^{-1}) in the PlusN treatment compared to the ZeroN treatment. In the nonirrigated environment, there was a nonsignificant ($P = 0.4441$) 0.89% reduction in seed weight (143 vs. 145 mg seed^{-1}). However, there was no significant ($P = 0.3489$, Table 3) difference in the effect of fertilizer N between irrigation environments.

Figure 4 shows a one-to-one graph of seed per square meter for the PlusN treatment compared to the ZeroN treatment across all management practices and irrigation environments. Of the 35 paired (ZeroN vs. PlusN) seed number samples (i.e., 70 observations with four replications each) only two data points fell below the one-to-one line. Within the irrigated environment, seed per square meter was significantly increased ($P < 0.0001$) by 9.5% (2623 vs. 2873 seed m^{-2}) in the PlusN treatment compared to the ZeroN treatment. Seed per square meter was significantly increased ($P < 0.0001$) in the nonirrigated environment by 16.2% (1963 vs. 2282 seed m^{-2}) in the PlusN treatment compared to the ZeroN treatment. The effect of fertilizer N on seed number per square meter was not significantly ($P = 0.2048$, Table 3) different between irrigation environments.

DISCUSSION

When averaged over all management practices (years, cultivars, herbicide treatments, and Mn treatments) and N treatments (PlusN and ZeroN), there was a large difference (3036 vs. 4346 kg m^{-2}) in seed yield between the nonirrigated and the irrigated environments. This, coupled with an analysis of the weather data over the 3 yr of the study (Table 2), clearly indicates that a differential water-deficit stress occurred between the two irrigation environments. The effect of irrigation on yield of soybean in the midsouthern USA is well documented (Heatherly, 1999c). It was expected that the large applications of fertilizer N applied in this study (>290 kg ha^{-1} each year) would affect nodulation and/or nodule activity (Harper and Gibson, 1984; Gibson and Harper, 1985). Although direct measurements of these parameters were not conducted in this study, the significant reductions in ureides between the PlusN and the ZeroN treatments (53.5% in the nonirrigated environment and 57.2% in the irrigated environment) clearly indicate a major effect on nodulation and/or nodule activity. The lower reduction in the nonirrigated environment compared to the irrigated environment can be attributed to the compounding negative effect of water deficit stress on N_2 fixation, and thereby ureide production.

Although ureides were significantly reduced in the PlusN treatment of both the irrigated and nonirrigated environments, N concentration was not significantly different between N treatments in the irrigated environment (3.37 vs. 3.40 g kg^{-1}). This indicates that N_2 fixation was sufficient to maintain the N levels of the plant under irrigation. However, the opposite was evident in the nonirrigated environment where the PlusN treatment resulted in significantly greater aboveground N concentration (2.97 g kg^{-1} in the ZeroN treatment vs. 3.28 g kg^{-1} in the PlusN treatment). This indicates that N_2 fixation was not fully meeting the vegetative N requirement of the soybean plants in a water deficit environment. These data support the hypothesis of Purcell and King (1996) that the uptake and assimilation of soil N is less sensitive to water deficits than is N_2 fixation.

In both the irrigated and nonirrigated environments, aboveground biomass was significantly increased with fertilizer N (14.1% irrigated and 16.7% nonirrigated, Fig. 1). Similarly, when total N was considered on an area basis (g of N m^{-2}, Fig. 2), there was a significant increase with fertilizer N of 12.8% in the irrigated environment and 28.1% in the nonirrigated environment. The more dramatic increase in N per square meter with N fertilization in the nonirrigated environment reflects the overall decrease in N concentration in the ZeroN treatment of the nonirrigated environment coupled with an increase in biomass in the PlusN treatment while maintaining the N concentration. A portion of the increases in biomass may reflect the lower carbon cost to the plant of assimilating N from the soil, thus freeing that carbon for additional growth. Nonetheless, the increased N accumulation in response to fertilizer N in both irrigated and nonirrigated environments (Fig. 2) indicates that N_2 fixation may be limiting overall growth. Again, the much larger increase in the nonirrigated environment supports the view that the uptake and assimilation of soil N are less sensitive to water deficits than is N_2 fixation.

In this study, seed yield in the PlusN treatment of the irrigated environment was 7.71% greater than the ZeroN treatment (Fig. 3). Seed yield was also significantly increased (15.53%) by the PlusN treatment in the nonirrigated environment (Fig. 3). The components of seed yield (seed weight and number of seed) in our study agree with those of Purcell and King (1996) and Sorensen and Penas (1978). They found that the majority of yield increase resulting from high rates of preplant N applied to soybean is attributable to increased number of seed. In our study, fertilizer N applied shortly after planting had little effect on seed weight in either the irrigated (2.19% increase) or the nonirrigated (0.89% decrease) environments. However, in both irrigation environments, the effect of fertilizer N on seed number was large (9.5% increase, irrigated and 16.2% increase, nonirrigated; Fig. 4). Linear relationships between seed number and crop growth rate have been widely reported for soybean and other grain crops (see discussion by Egli, 1998). The increases in biomass from fertilizer N found in this study represent an increased growth rate, and would therefore support an increased seed number (Egli, 1998). These results indicate that soybean yield increases attributable to early-season N application result from enhancement of plant processes that occur early in reproductive development rather than during the seedfill period. Thus, N from N_2 fixation in soybean may be insufficient early in the season for maximum yield potential regardless of irrigation environment. Muchow and Sinclair (1986) concluded that N input was a ''major constraint'' to high soybean yields based on analysis of crop simulations. However, the large effect of fertilizer N on seed number observed in this study and others (Sorensen and Penas, 1978; Purcell and King, 1996) indicates that late-season applications of N to soybean may not compensate for suboptimal N supply (limited by N_2 fixation) that occur early in the growing season since they would be too late to increase number of seed.

In our study, seed yield was increased with the addition of fertilizer N in both the irrigated and nonirrigated environments (7.71 and 15.53%, respectively; Fig. 3) across a wide range of management practices (years, cultivars, herbicide treatments, and Mn treatments). Nonetheless, using fertilizer N at the rate used in this study was not economical. Even though yield increases resulted from addition of fertilizer N, fertilizer costs far exceeded the return from the increased yield. However, these results indicate that there is a yield limitation imposed by N_2 fixation in both irrigated and nonirrigated environments, and that the uptake and assimilation of soil N is less sensitive to water deficits than is N_2 fixation. The much greater yield increase from fertilizer N in the nonirrigated environment compared to the irrigated environment highlights the sensitivity of N_2 fixation to water deficit stress. Understanding the processes leading to the sensitivity of N_2 fixation during water deficit stress may allow development or exploitation of alternative pathways within the plant that will increase soybean yields in nonirrigated environments without producers expending additional resources.

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